Cloning and Characterization of a *Bacteroides* Conjugal Tetracycline-Erythromycin Resistance Element by Using a Shuttle Cosmid Vector

NADJA B. SHOEMAKER,* ROBERT D. BARBER, AND ABIGAIL A. SALYERS

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Received 10 August 1988/Accepted 1 December 1988

The *Bacteroides* conjugal tetracycline resistance (Tc^r) elements appear not to be plasmids. In many cases, resistance to erythromycin (Em^r) is cotransferred with Tc^r. Using a newly constructed shuttle cosmid, pNJR1, we cloned 44 to 50 kilobase pairs of a conjugal Tc^r Em^r element on overlapping cosmid clones. Cosmid libraries were made in *Escherichia coli* with DNA from the original clinical *Bacteroides thetaiotaomicron* DOT strain containing Tc^r Em^r-DOT or from a *Bacteroides uniformis* Tc^r Em^r-DOT transconjugant strain. The cosmid clones were mobilized from *E. coli* into *B. uniformis* in groups of 10 to 20 per filter mating, with selection for Tc^r or Em^r transconjugants. The Tc^r and Em^r genes were cloned both separately and together on 30-kilobase-pair fragments. Several of the Tc^r clones also contained transfer genes that permitted self-transfer of the cosmid from *B. uniformis* donors to *E. coli* or *B. uniformis* recipients. Neither the Tc^r nor the Em^r gene conferred resistance on *E. coli*, and the transfer-proficient clones did not self-transfer out of *E. coli*. Southern blot analysis was used to compare DNA from independently isolated *Bacteroides* strains carrying conjugal Tc^r or Tc^r Em^r elements and their respective *B. uniformis* transconjugants. Results of these analyses indicate that there are large regions of homology, including regions outside the Tc^r and Em^r genes, but that the elements are not identical. Some Tc^r clones contained a region which hybridized to chromosomal DNA from the wild-type *B. uniformis* recipient strain that did not carry the Tc^r Em^r-DOT element. This region of homology appeared not to be a junction fragment. It was not required in a *Bacteroides* recipient for successful transfer of the Tc^r Em^r element. Although we are not sure we have cloned a junction fragment between the Tc^r Em^r-DOT element and the *B. uniformis* chromosome, the preliminary function and restriction map appears to be linear.

Bacteroides spp. appear to carry two different types of conjugal elements. One type is exemplified by the conjugal erythromycin resistance (Em^r) plasmids pBF4 and pBI136 (24, 32). The same Em^r gene has also been found on mobilizable plasmids such as pBFTM10 and pCP1 (6, 29). In all cases, the Em^r gene is flanked by the same directly repeated insertion sequences (7, 13, 14, 25). No *Bacteroides* plasmids have yet been described that carry a *Bacteroides* tetracycline resistance (Tc^r) gene. Instead, transferable Tc^r genes are associated with elements that are thought to be chromosomal, similar to those first described in the streptococci (22). Some of these elements cotransfer Tc^r and Em^r, whereas others transfer Tc^r alone (12, 16). These conjugal Tc^r and Tc^r Em^r elements can also mobilize coresident plasmids to *Bacteroides* or *Escherichia coli* recipients (18, 30). Although *Bacteroides* Em^r plasmids have been extensively characterized, none of the *Bacteroides* Tc^r or Tc^r Em^r elements have previously been cloned. Therefore, no information on the structure of these elements is available, nor is it known whether the Tc^r and Tc^r Em^r elements found in different strains are related.

Since the Em^r gene found in *Bacteroides* plasmids does not confer resistance on *E. coli* (6, 19), it was also possible that the *Bacteroides* Tc^r gene would not be expressed in *E. coli*. Therefore, to isolate the Tc^r and Em^r genes from *Bacteroides* strains, we decided to do the initial cloning in *E. coli* and then transfer the clones to *Bacteroides* recipients to select for expression of the resistances. In this paper, we describe a shuttle cosmid, pNJR1, that we constructed for this purpose. Using the shuttle cosmid, we have cloned not

only the Tc^r and Em^r genes from a conjugal Tc^r Em^r strain of *Bacteroides* but also large regions of the element including transfer genes. The DNA was obtained from *Bacteroides thetaiotaomicron* DOT, a strain that cotransfers Tc^r and Em^r to *Bacteroides* recipients. This element is designated Tc^r Em^r-DOT. DNA was also cloned from a *Bacteroides uniformis* transconjugant that had received the Tc^r Em^r-DOT element in a mating with *B. thetaiotaomicron* DOT. The clones obtained from these strains have enabled us to deduce part of the structure of the element and to ascertain whether this element is closely related to the conjugal Tc^r elements from *Bacteroides fragilis* V479 (Tc^r-V479) and *B. fragilis* ERL (Tc^r-ERL) and to the conjugal Tc^r Em^r elements from *B. fragilis* ERL (Tc^r Em^r-ERL), *B. fragilis* 12256 (Tc^r Em^r-12256), and *B. fragilis* CEST (Tc^r Em^r-CEST).

MATERIALS AND METHODS

Strains and plasmids. Strains and plasmids used in this study are listed in Table 1. The *E. coli* strains were grown on Luria broth (LB) or agar, and the *Bacteroides* strains were maintained on prereduced Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-yeast extract-glucose (TYG) broth or agar as described previously (19). Plasmid preparations for both organisms were made by using the Ish-Horowitz modification of the Birnboim and Doly method (10).

Construction of cosmids. Chromosomal DNA was prepared according to Saito and Miura (15). Freshly prepared chromosomal DNA from *B. thetaiotaomicron* DOT and *B. uniformis* 1006 was partially digested with *Sau*3A under conditions that gave a visible widening of the chromosomal band on a stained agarose gel but did not give extensive

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant phenotype ^a	Description or reference
<i>E. coli</i> strains		
HB101	RecA ⁻ Str ^r	1
EM24	RecA ⁻ Str ^r	RecA ⁻ derivative of LE392 (10, 18)
S17-1	RecA ⁻ Tp ^r Sm ^r (ORP42-Tc::Mu-Kn::Tn7) ^b	IncP RP4 inserted in chromosome (23)
<i>Bacteroides</i> strains		
<i>B. uniformis</i>		
1001	Rif ^r	Spontaneous Rif ^r mutant of <i>B. uniformis</i> 0061 (19)
1100	Thy ⁻ Tpm ^r	Spontaneous Thy ⁻ mutation in <i>B. uniformis</i> 0061 that causes the strain to be Tpm ^r (18)
1006	Rif ^r Tc ^r Em ^r	<i>B. uniformis</i> 1001 Tc ^r Em ^r transconjugant from <i>B. thetaiotaomicron</i> DOT (21)
1002	Rif ^r Tc ^r	<i>B. uniformis</i> 1001 Tc ^r transconjugant from <i>B. fragilis</i> V479 (21)
1003	Rif ^r Tc ^r Em ^r	<i>B. uniformis</i> 1001 Tc ^r Em ^r transconjugant from <i>B. fragilis</i> CEST (21)
1004	Rif ^r Tc ^r	<i>B. uniformis</i> 1001 Tc ^r transconjugant from <i>B. fragilis</i> ERL (18, 20, 21)
1005	Rif ^r Tc ^r Em ^r	<i>B. uniformis</i> 1001 Tc ^r Em ^r transconjugant from <i>B. fragilis</i> ERL (21)
1008	Rif ^r Tc ^r Em ^r	<i>B. uniformis</i> 1001 Tc ^r Em ^r transconjugant from <i>B. fragilis</i> 12256 (21)
<i>B. fragilis</i>		
CEST	Tc ^r Em ^r	Clinical isolate containing conjugal Tc ^r Em ^r -CEST (21)
ERL	Tc ^r Em ^r	Clinical isolate containing conjugal Tc ^r -ERL and Tc ^r Em ^r -ERL (18, 21)
12256	Tc ^r Em ^r Ap ^r	Clinical isolate containing conjugal Tc ^r Em ^r -12256 (11)
V479C	Tc ^r	Clinical isolate, cured of pBF4, containing conjugal Tc ^r -V479 (19, 26)
<i>B. thetaiotaomicron</i>		
5482A	Nal ^r	Type strain (Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg).
DOT	Tc ^r Em ^r Ap ^r	Clinical isolate containing conjugal Tc ^r Em ^r -DOT (21)
Plasmids		
R751	Tp ^r Tra ⁺	IncP plasmid used to mobilize vectors from <i>E. coli</i> to <i>Bacteroides</i> recipients (19)
pB8-51		4.2-kbp <i>Bacteroides</i> cryptic plasmid, used to construct chimeric shuttle vectors (18, 19)
pJRD215	Kn ^r Sm ^r Mob ⁺	Broad-host-range RSF1010-based cosmid vector; mobilized at high frequencies by IncP plasmids (3)
pNJR1	Kn ^r Sm ^r Mob ⁺ (Rep ⁺ Mob ⁻)	Shuttle cosmid, containing pJRD215 and pB8-51; no selectable <i>Bacteroides</i> phenotypes; used to clone <i>Bacteroides</i> Tc ^r and Em ^r genes and transfer functions (Fig. 1)
pNJR5	Kn ^r Mob ⁺ (Em ^r Rep ⁺ Mob ⁻)	<i>Bacteroides</i> Em ^r gene from Tn4400 on pBFTM10 (14, 29) cloned into <i>EcoRI</i> - <i>Clal</i> region of pNJR1; expresses Em ^r in <i>Bacteroides</i> recipients (this study)
pFD214	Ap ^r (Em ^s)	pUC19-based shuttle vector containing promoterless, sequenced, 0.8-kbp <i>Bacteroides</i> gene (25)
pBS9	Ap ^r	pBR328-based vector containing 0.9-kbp <i>Clal</i> -to- <i>EcoRI</i> fragment of Tn4351 (see Fig. 2D) (28)

^a Abbreviations for bacterial phenotypes Str^r, streptomycin resistance; Rif^r, rifampin resistance; Thy⁻, thymidine requiring; Tpm^r, trimethoprim resistance due to Thy⁻ phenotype. Abbreviations for plasmid, transposon, or conjugal element phenotypes: Tp^r, trimethoprim resistance; Sm^r, streptomycin resistance; Kn^r, kanamycin resistance; Tc^r, tetracycline resistance; Em^r, erythromycin resistance; Ap^r, ampicillin resistance; Tra⁺, capable of self-transfer; Mob⁺, can (+) and cannot (-) be mobilized; Rep⁺, can replicate. Within parentheses for plasmid phenotypes are phenotypes that are expressed in *Bacteroides* recipients; outside the parentheses are phenotypes expressed in *E. coli*.

^b IncP plasmid RP4 inserted into the S17 chromosome via bacteriophage Mu. Tn7 was inserted in the remaining Kn^r and provides the Tp^r Sm^r phenotype. RP4 provides transfer genes necessary to mobilize RSF1010 shuttle vectors (e.g., pNJR1).

digestion. The digested DNA was cloned into the *Bam*HI site of pNJR1 as described by Maniatis et al. (10). The ratio of insert to vector was approximately 1:10. For construction of pNJR1, see Fig. 1 and Results. The cosmids were packaged in λ heads by an in vitro packaging system (2). The packaged cosmids were used to transfect *E. coli* EM24 or S17-1 (Table 1).

From previous experience with cloning *Bacteroides* DNA, we knew that substantial loss of cloned inserts can occur in *E. coli*, even in a *recA* background. This deletion of insert DNA increases with every transfer and regrowth of the *E. coli* cosmid library. To minimize loss of cloned inserts, we prepared about 3 ml of transfected *E. coli* cells and then froze all but 0.1 ml in 15% glycerol and stored it at -80°C. Dilutions of the 0.1-ml portion were plated onto LB-kanamycin plates to determine the concentration of kanamycin-resistant (Kn^r) cells in the cosmid-transfected *E. coli* culture.

Plasmid preparations were made from 20 Kn^r isolates, and the percentage of intact 40- to 46-kilobase-pair (kbp) cosmid clones was determined. Once the concentration of cosmid clones was known, the frozen culture was thawed and diluted appropriately so that about 10- to 20 full-sized cosmid clones were inoculated into each 3 ml of LB culture for use as donors in the matings described below.

Nitrocellulose filter matings. The filter matings were done as previously described (18, 20) except that portions of the infected *E. coli* culture, containing approximately 10 to 20 full-sized (40- to 46-kbp) cosmids, were inoculated into 3 ml of LB plus kanamycin (75 μ g/ml). The cultures, usually 40 to 50 at a time, were allowed to grow overnight with vigorous aeration. Fresh cultures were inoculated (1/50 dilution) into LB-kanamycin, and the cultures were allowed to grow to about 0.5×10^8 to 1×10^8 cells per ml. Portions (0.1 ml) of the mixed *E. coli* donors were mixed with 0.4 ml of *B.*

uniformis 1001 recipients that had been grown to a similar cell density in anaerobic TYG. If EM24 was the cosmid host, 0.1 ml of a second *E. coli* donor culture, containing R751 to mobilize the cosmids, was added to the mating mixture. The cells were centrifuged in a microfuge for 2 min, washed once with TYG, resuspended in 30 μ l of TYG, and spotted on a 13-mm HAWP 0.45- μ m-pore-size nitrocellulose filter (Millipore Corp., Bedford, Mass.) on the surface of a TYG agar plate. There were usually nine filters per plate. The plates were incubated aerobically for about 17 h at 37°C. Each filter was resuspended in 1 ml of TYG, and cells were diluted and plated on selective TYG plates containing gentamicin (200 μ g/ml) and erythromycin (10 μ g/ml) or tetracycline (3 μ g/ml). The plates were incubated anaerobically at 37°C for 48 h. The entire mating mixture was always plated because some of the cosmids grow more slowly than others in *E. coli* because of their inserts and therefore can be a low percentage of the mating mixture. Since pNJR1 type vectors (pNJR5 and pE5-2) are mobilized by IncP plasmids to *B. uniformis* 1001 recipients at a frequency of 10^{-3} (19) and since we can easily detect frequencies as low as 10^{-8} to 10^{-9} , we should be able to isolate transconjugants representing even the slow growers from a mixture of 10 to 20 cosmids.

The *B. uniformis* 1001 Tc^r and Em^r transconjugants were streaked for purity and inoculated into chopped-meat anaerobic medium (Carr-Scarborough Microbiology, Decatur, Ga.) containing tetracycline (1 μ g/ml) or erythromycin (10 μ g/ml). Frozen stock cultures of the fresh chopped-meat cultures plus 15% glycerol were made and stored at -80°C. Viability and stability of plasmids in our *Bacteroides* cultures maintained at -80°C has been excellent. Tetracycline or erythromycin was always present in the growth medium unless indicated. *B. uniformis* 1100 (thymidine requiring [Thy⁻] and thus trimethoprim resistant [Tpm^r]) transconjugants from *B. uniformis* 1001 (rifampin resistant [Rif^r]) donors were selected on TYG containing 100 μ g each of thymidine and trimethoprim per ml as well as erythromycin or tetracycline. The *B. uniformis* 1100 transconjugants were also rifampin sensitive (Rif^s).

B. uniformis-to-*B. uniformis* matings of some transfer-proficient cosmids are done anaerobically with selection for Tpm^r and Tc^r (18). *B. uniformis*-to-*B. thetaiotaomicron* matings were done the same way except that selection was for nalidixic acid resistance (Nal^r) and Tc^r. In some cases, matings from *B. uniformis* (or *B. thetaiotaomicron*) to *E. coli* were done. The procedure has been described previously (18). Since the only marker on the cosmids that expresses in *E. coli* is kanamycin resistance, *E. coli* transconjugants were selected on aerobic LB plates containing 75 μ g of kanamycin per ml.

Subcloning. The region of the cosmid clones containing the *Bacteroides* Tc^r gene was localized by subcloning fragments of the cosmid clones into pNJR1 or pNJR5. *E. coli* strains were transformed by using frozen competent cells as previously described (19), with selection on LB-kanamycin plates. Subclones were then mobilized into *B. uniformis* 1001 to test the *Bacteroides* resistances, using the mobilization procedure described previously (18).

Agarose gels and Southern blot analysis. Chromosomal and plasmid DNAs were digested with restriction enzymes according to the instructions of the manufacturer (Bethesda Research Laboratories, Inc., Rockville, Md., or Boehringer-Mannheim Biochemicals, Indianapolis, Ind.). Digested DNA was loaded on agarose slab gels (0.8 to 1%) in 4 $\times$ GGB (0.16 M Tris, 0.08 M sodium acetate, 8 mM EDTA [pH 8.3]) and electrophoresed for 15 to 17 h at 50 V. The gels were stained

FIG. 1. Shuttle cosmid pNJR1. Davison et al. (3) constructed a mobilizable, broad-host-range cosmid vector, called pJRD215, from the IncQ plasmid RSF1010. To enable this vector to replicate in *Bacteroides* hosts, the cryptic 4.2-kbp *Bacteroides* plasmid pB8-51 (19) was cut with *HincII* and cloned into the unique *HpaI* site of pJRD215. The resultant 14.4-kbp chimeric shuttle cosmid, pNJR1, can be mobilized to *Bacteroides* recipients from *E. coli* by IncP plasmids and is capable of replicating in *Bacteroides* recipients. pNJR1 contains no selectable *Bacteroides* markers and cannot transfer itself or be mobilized out of *Bacteroides* hosts.

with ethidium bromide and photographed. For Southern blot analysis, the DNA was transferred to Millipore HAHY nitrocellulose paper (10). Nick translation of the probes with [³²P]dCTP, hybridization, and washing of the filters were done as described previously (17).

RESULTS

Construction of cosmid clones. The shuttle cosmid pNJR1, which can replicate in both *E. coli* and *Bacteroides* hosts, was constructed as shown in Fig. 1. The cryptic *Bacteroides* plasmid pB8-51 (19) was digested with *HincII* and cloned into the *HpaI* site of the broad-host-range RSF1010 cosmid vector pJRD215 (3) to provide the origin of replication in *Bacteroides* spp. Because we wanted to use pNJR1 to clone either Tc^r or Em^r, pNJR1 has no selectable markers that function in *Bacteroides* hosts. Therefore, to determine the transfer frequency of this cosmid and test its stability in *Bacteroides* recipients, the erythromycin resistance from the *Bacteroides* plasmid pBFTM10 (29) was cloned into the *EcoRI*-*ClaI* region of pNJR1 to form pNJR5 (not shown). The IncP plasmid RP4, inserted into the chromosome in strain S17-1 (Table 1), mobilized pNJR5 to *Bacteroides* recipients by filter matings at a frequency of 10^{-3} transconjugants per recipient. pNJR5 was very stable in the *Bacteroides* recipients even when cells were grown under nonselective conditions. When *E. coli* or *Bacteroides* strains carrying this plasmid were grown for 20 generations in LB or TYG, respectively, without antibiotic selection and 200 colonies were screened for markers on the plasmid, 100% of the colonies still contained the plasmid. Restriction digests of plasmid preparations confirmed that no deletions or rearrangements had occurred.

TABLE 2. Tc^r Em^r-DOT cosmid clones isolated in *B. uniformis* 1001^a

Source of cloned DNA	Phenotype	Cosmids isolated ^b	
		No. of isolates	Insert size (kbp)
<i>B. thetaiotaomicron</i> DOT	Tc ^r Em ^r	3	27-33 (3)
	Tc ^r	3	30 (2), 19 (1)
<i>B. uniformis</i> 1006	Tc ^r Em ^r	1	30
	Tc ^r	5	30 (3), 16 (2)
	Em ^r	6	25 (2), 12 (1), 6 (3)

^a Cosmids were prepared in *E. coli* (see text) and mobilized by conjugation into *B. uniformis* 1001 for selection of *Bacteroides* phenotype Tc^r or Em^r.

^b Full cosmid size for lambda packaging would contain 25 to 30 kbp of insert DNA. The number of isolated cosmids which contained the indicated insert size is given in parentheses.

pNJR1 was used to clone both the tetracycline and erythromycin resistances of the apparently nonplasmid conjugal Tc^r Em^r-DOT element. The pNJR1 cosmid clones were prepared from total DNA of the clinical isolate, *B. thetaiotaomicron* DOT, and from a *B. uniformis* transconjugant (1006) that contained the conjugative Tc^r Em^r-DOT element as described in Materials and Methods. In some cases, as few as 10 to 20% of the *E. coli* hosts that carried cosmids still had intact full-sized (40- to 44-kbp) cosmid inserts. However, it was possible to obtain cosmid preparations in which 40 to 60% of the cosmid clones were full sized, and only such preparations were used for the matings. A total of 400 to 800 potential cosmids (40 mating filters) were tested in each experiment, and a total of 3,000 potential cosmid clones were transferred by conjugation to *B. uniformis* 1001. Of these, 12 clones carrying Tc^r, Em^r, or both were isolated from *B. uniformis* 1006 DNA and 6 were isolated from *B. thetaiotaomicron* DOT DNA. The properties of the cosmid clones are summarized in Table 2. No two cosmid clones were isolated from the same mating mixture of 10 to 20 potential cosmids. Note that several of the cosmid clones contained inserts smaller than the 25 to 30 kbp predicted for a full-sized insert. These cosmids had probably sustained deletions. The Em^r clones were particularly prone to deletions.

All of the cosmid clones were isolated as plasmids and transformed into *E. coli*. No expression of the Tc^r or Em^r genes was detected in the *E. coli* transformants. Several of the larger cosmid clones exhibited some instability in *E. coli* hosts, whereas others were relatively stable, with no obvious deletions occurring. The failure to detect Tc^r or Em^r in *E. coli* was not due to deletion or mutation of the resistance genes, because when these clones were mobilized back into *Bacteroides*, they still conferred resistance on the *Bacteroides* recipients.

Transfer functions found on Tc^r cosmid clones. The cosmid pNJR1 can replicate in *Bacteroides* hosts, but it has no self-transfer functions. RSF1010 is not mobilized by any of the known *Bacteroides* conjugal elements (30). Although pB8-51 contains a mobilization region, we have shown previously that when cloned into a shuttle vector in its *Hinc*II site (e.g., pE5-2), it cannot be mobilized out of *Bacteroides* donors (18, 30). Thus, it should be possible to screen clones in pNJR1 to locate regions that code for transfer functions. To confirm that pNJR1 itself was not mobilized, we tested transfer of pNJR5, which is pNJR1 containing the *Bacteroides* Em^r gene, out of *B. uniformis* 1001 (Table 3). No transfer was detected. Accordingly, all of

TABLE 3. Self-transfer of Tc^r cosmids from *B. uniformis* 1001 donors to *E. coli* or *B. uniformis* recipients

Cosmid in <i>B. uniformis</i> 1001 donor ^a	Frequency of transfer to ^b :	
	HB101 (Kn ^r)	1100 (Tpm ^r Tc ^r)
p6T5	1.5×10^{-4} – 2.7×10^{-4c}	2×10^{-7} – 12×10^{-7}
pD2-T1	1.9×10^{-3} – 4.0×10^{-3}	3.8×10^{-7} – 230×10^{-7}
pD2-T2	2.3×10^{-4} – 3×10^{-4c}	$<3 \times 10^{-9}$
pD2-TE1	1×10^{-8} – 3×10^{-8}	$<10^{-8}$
p6TE	1×10^{-8} – 3×10^{-8}	$<10^{-8}$
pNJR5	$<10^{-9}$	$<10^{-8}$

^a Cosmid prefixes 6 and D2 denote that the sources of the insert DNA were *B. uniformis* 1006 and *B. thetaiotaomicron*, DOT, respectively. All of these cosmids contained at least 30 kbp of insert DNA. pNJR5 was the control cosmid, containing Em^r but no cloned DNA from Tc^r Em^r-DOT (Table 1).

^b Cosmid transconjugants were selected on aerobic LB-kanamycin plates in *E. coli* HB101 and anaerobic TYG plates containing trimethoprim and tetracycline in *B. uniformis* 1100. Possible pNJR5 transconjugants were selected on TYG containing erythromycin instead of tetracycline. Nitrocellulose filter matings were done as described previously (18). Frequencies are expressed as transconjugants per recipient at the end of the mating. Frequency ranges are for three separate experiments.

^c Approximately 90% of the HB101 transconjugants contained deleted forms of the original cosmid.

the cosmid clones were tested for ability to transfer out of *B. uniformis* 1001 to the nearly isogenic strain *B. uniformis* 1100 or to *E. coli* recipients.

Three of the large Tc^r cosmid clones, one from *B. uniformis* 1006 (p6T5) and two from *B. thetaiotaomicron* DOT (pD2-T1 and pD2-T2), transferred to *E. coli* recipients at frequencies of 10^{-4} to 10^{-3} (Table 3). Two Tc^r Em^r cosmid clones, p6TE (BU1006) and pD2-TE1 (*B. thetaiotaomicron* DOT), transferred at very low frequencies. None of the other cosmid clones transferred to *E. coli* at detectable frequencies ($<10^{-9}$). When *B. uniformis* 1100 was used instead of *E. coli* as the recipient, however, the cosmid transfer frequencies were considerably lower. Also, whereas the transfer frequencies of the cosmid clones to *E. coli* recipients were very reproducible, the transfer frequencies to *B. uniformis* 1100 varied by as much as 100-fold between experiments. pD2-T2 was never transferred successfully to *B. uniformis* 1100. Analysis of plasmid preparations from the *E. coli* transconjugants showed that 90% of the pD2-T2 cosmids had large deletions (data not shown). More than 50% of the p6T5 transconjugants contained deleted forms of the cosmid clone, but pD2-T1 appeared to transfer intact. All of the cosmid clones in the *B. uniformis* 1100 transconjugants appeared to be intact, with no obvious deletions or rearrangements detectable by restriction digest analysis. The cosmid clones p6T5 and pD2-T2 were much more stable in *E. coli*, especially in HB101, when introduced by transformation rather than conjugation. More than 90% of the transformants appeared to contain intact cosmid clones (data not shown). Therefore, the deletions probably occurred during the transfer process.

Since it was possible that the transfer-proficient cosmids were being mobilized by some chromosomal element in *B. uniformis* 1001, we also mated our most stable transfer-proficient clones, 6T5 and D2T1, into *B. thetaiotaomicron* 5482 and checked for transfer of the cosmids from strain 5482 into *E. coli*. Transfer frequencies were lower than those seen in *B. uniformis* matings (10^{-7} to 10^{-6} rather than 10^{-4} to 10^{-3}), but transfer of the element was easily detectable.

The transfer genes on the Tc^r clones did not function in *E. coli* hosts. The cosmids could not transfer themselves from *E. coli* to either *E. coli* or *B. uniformis* recipients. The

mobilization of transfer-minus cosmids by R751 in triparental matings from *E. coli* to *B. uniformis* 1001 was usually equivalent to that seen for pNJR5 alone (10^{-4} to 10^{-3}), regardless of size. However, the three conjugal Tc^r clones were always transferred at much lower frequencies (10^7 to 10^8). The resultant *B. uniformis* 1001 transconjugants appeared to contain intact cosmids. These results provide further evidence for a destabilization of Tc^r cosmids containing the *Bacteroides* transfer genes during the conjugation process.

Localization of the Em^r-DOT gene on cosmids and relationship of this gene to the Em^r region in other Tc^r Em^r elements. Southern blot analysis had shown previously that the Em^r gene on the *Bacteroides* transposon Tn4351 hybridized to DNA from *B. uniformis* 1001 strains that contained different conjugal Tc^r Em^r elements, including Tc^r Em^r-DOT (N. B. Shoemaker, unpublished data). Similar reports for other conjugal Tc^r Em^r elements have been made (see reviews in references 12 and 16). Therefore, we used two probes containing the Em^r gene found on Tn4351 (13, 17) to help us localize the Em^r gene on our clones and to compare the hybridization patterns of the enzyme-digested DNA from *B. uniformis* 1001 strains containing different conjugal Tc^r Em^r elements by Southern blot analysis. One of these probes, pFD214 (25), had the Em^r gene on a 0.8-kbp fragment with virtually no other DNA. The second contained the 3.8-kbp *EcoRI* fragment from Tn4351. This fragment includes not only the Em^r gene but also most of IS4351 and the cryptic tetracycline resistance gene (*Tc^r) that is adjacent to the Em^r gene on Tn4351 (5, 28). The cosmid clones and the total DNA from the *B. uniformis* 1001 Tc^r Em^r strains (Table 1) were digested with *HindIII*, which should cut once within the Em^r gene, and with *EcoRI* or *HincII*, which should cut outside the Em^r gene. Southern blot analysis of chromosomal DNA from three *B. uniformis* 1001 strains containing different conjugal Tc^r Em^r elements is shown in Fig. 2. Similar patterns were seen in all hosts. The probe, pFD214, which contained only the 0.8-kbp Em^r gene, hybridized to two *HindIII* fragments and one *EcoRI* fragment (Fig. 2B). This is the same pattern seen when this fragment is used to probe Tn4351 (17). However, the entire 3.8-kbp *EcoRI* fragment of Tn4351 (Fig. 2D) hybridized to an additional *EcoRI* fragment (Fig. 2A).

The additional homology could be shown to be at least partially contained on the 0.9-kbp *ClaI*-to-*EcoRI* fragment of Tn4351 on probe pBS9 (Fig. 2D), which includes part of an aerobic tetracycline resistance (*Tc^r) that is cryptic in *Bacteroides* and is expressed only in aerobically grown *E. coli* (5, 28). The *ClaI*-*EcoRI* fragment hybridized to the larger *HindIII* fragment (6.5 kbp), which also hybridized to the Em^r probe (Fig. 2B), and to the smaller *EcoRI* fragment (7 kbp), which did not hybridize to the Em^r probe (Fig. 2C). Thus, the extra homology to Tn4351 appears to be contiguous to the Em^r gene.

Although several of the Em^r and Tc^r Em^r clones included the 7-kbp *EcoRI* region that hybridized to the probes containing all or part of the *Tc^r gene from Tn4351, none of them expressed *Tc^r in *E. coli*. Thus, the homology is either to a defective *Tc^r gene or to the region outside the *Tc^r gene on the 0.9-kbp *ClaI*-*EcoRI* fragment of Tn4351. Probes containing the insertion element on Tn4351, IS4351, did not hybridize to DNA from *B. uniformis* 1001 or to DNA from any of the 1001 or clinical strains containing the conjugal Tc^r Em^r elements (data not shown). This finding is in agreement with reports from other laboratories that strains with chromo-

FIG. 2. Autoradiogram illustrating homology of *B. uniformis* 1001 strains containing conjugal Tc^r Em^r elements to Tn4351 probes. The total DNA from three 1001 strains containing separately isolated conjugal Tc^r Em^r elements were digested with *HindIII* and *EcoRI*. *B. uniformis* 1005 (Tc^r Em^r-ERL), 1006 (Tc^r Em^r-DOT), and 1008 (Tc^r Em^r-12256) are in lanes 1, 2, and 3, respectively. Results for strain 1003 (Tc^r Em^r-CEST), not included on this autoradiogram, were identical to those for 1006. The Southern blots of the digested DNAs were probed with ³²P-labeled probes containing the 3.8-kbp *EcoRI* fragment of Tn4351 (A), an 0.8-kbp segment containing the Em^r gene from Tn4351 on pFD214 (B), and the 0.9-kbp *ClaI*-to-*EcoRI* fragment of Tn4351 on pBS9 (C). The Tn4351 portions of the probes are indicated (D). The Em^r gene of Tn4351 is nearly identical to the Em^r gene of Tn4551 by DNA sequence data (13, 25). The sizes (in kilobase pairs) of *HindIII* λ standards are indicated on the right. The positions of the two *HindIII* fragments (a and b) and the two *EcoRI* fragments (c and d) that hybridize to the entire *EcoRI* fragment of Tn4351, shown in panel A, are indicated in all three panels. No hybridization was seen to probes containing IS4351 (not shown).

somal Em^r genes do not carry the insertion elements that flank the Em^r gene in the plasmids (12).

Localization of the region containing the Tc^r gene and comparison of this region of the Tc^r-DOT element with that of five *Bacteroides* conjugal Tc^r and Tc^r Em^r elements. The Tc^r cosmid clones were digested with a variety of restriction enzymes, and the digest patterns were compared on agarose gels to look for common fragments that might localize the Tc^r region. One of the enzymes that gave several common fragments internal to the cloned DNA was *EcoRV*. All of the Tc^r clones contained a 1.1-kbp *EcoRV* fragment, and most of the clones also contained a 2.4- and 2.1-kbp *EcoRV* fragment (Fig. 3). Subcloning experiments indicated that the *Bacteroi*-

FIG. 3. Agarose gel of *EcoRV*-digested Tc^r-DOT cosmid clones. Plasmid preparations were made from several of the Tc^r and Tc^r Em^r-DOT clones in pNJR1, and their *EcoRV* digests were run on an 0.8% agarose gel. Lane 9 contains *HindIII* λ and *HaeII* ϕ X174 standards. Asterisks indicate positions of the internal pNJR1 *EcoRV* fragments. Three prevalent *EcoRV* bands that are internal to the DNA inserted in pNJR1 (2.4, 2.1, and 1.1 kbp) are indicated by arrowheads. Note that all of the clones contain the 1.1-kbp *EcoRV* fragment. p6T3 (lane 1), p6T4, (lane 2), and pD2-T3 (lanes 8 and 10) are Tc^r clones with inserts of 16 to 19 kbp; p6T5 (lane 3) and pD2-T2 (lane 4) are Tc^r clones with inserts of 25 to 30 kbp that contain transfer functions; p6TE (lane 5), pD2-TE2 (lane 6), and pD2-TE3 (lane 7) are Tc^r Em^r clones with inserts of 25 to 30 kbp. All of the plasmids were isolated from *B. uniformis* 1001 hosts except pD2-T3 (lane 10), which was prepared from HB101 to be used as a probe in Southern blot analysis.

des Tc^r gene was located within a 5-kbp fragment that included the smallest *EcoRV* fragment.

To determine whether the Tc^r gene and the region surrounding it were conserved in different Tc^r elements, the smallest Tc^r clone (16-kbp insert) from *B. thetaiotaomicron* DOT, pD2-T3 (Fig. 3, lanes 8 and 10), which had the 1.1- and 2.1-kbp but not the 2.4-kbp *EcoRV* fragments, was isolated from an HB101 host and used as a probe in a Southern blot analysis of five different conjugal Tc^r or Tc^r Em^r elements. The autoradiogram of the *EcoRV*-digested DNA from five independent clinical isolates that contained a conjugal Tc^r or Tc^r Em^r element and their respective *B. uniformis* 1001 transconjugants probed on Southern blots with ³²P-labeled pD2-T3 is shown in Fig. 4. All of the Tc^r or Tc^r Em^r strains contained *EcoRV* fragments that hybridized to the probe. Although pD2-T3 did not contain an intact 2.4-kbp *EcoRV* fragment (Fig. 3, lane 10), it hybridized to a 2.4-kbp fragment in the parental DOT strain and the transconjugant 1006 (Fig. 4, lanes 4 and 4a, respectively).

The only strains that did not have the 2.4- and 2.1-kbp fragments were *B. fragilis* V479C and the corresponding transconjugant, *B. uniformis* 1002 (Fig. 4, lanes 1 and 1a). The conjugal Tc^r element from *B. fragilis* V479C had strong regions of homology to the probe, including a 1.1-kbp *EcoRV* fragment, but the restriction pattern was quite different. In addition to the small *EcoRV* fragments, all of the strains had one or two large (16- to 23-kbp) *EcoRV* fragments that hybridized to pD2-T3. A larger Tc^r clone (p6T5) from *B. uniformis* 1006, p6T5 (Fig. 3, lane 3), which was transfer proficient (Table 3) and contained an additional 18- to 20-kbp *EcoRV* fragment, gave the same hybridization pattern as did

pD2-T3 except that it hybridized to an additional 18- to 20-kbp *EcoRV* band in all strains, including *B. fragilis* V479C and *B. uniformis* 1002 (data not shown). Therefore, there is definitely strong homology between all of the conjugal Tc^r elements and regions of the Tc^r-DOT clones which include the Tc^r gene, the transfer regions, or both. Note that the 2.4- and 2.1-kbp *EcoRV* bands were not present in pD2-TE2 and pD2-TE3 (Fig. 3, lanes 6 and 7) and are probably outside the Tc^r gene (see below).

Although pD2-T3 was isolated from a *B. thetaiotaomicron* strain, it contained a region that hybridized to the *B. uniformis* 1001 wild-type strain (Fig. 4, lane 6). There was strong homology to a large 16- to 18-kbp fragment and to a band which migrated at around 3.8 kbp. Note that the 3.8-kbp band was not present in *B. uniformis* 1004 (lane 2a) or 1005 (lane 2b). This result could indicate that the 3.8-kbp band contains an insertion site for the elements; if so, Tc^r-ERL and Tc^r Em^r-ERL are inserted in sites on the chromosome different from those of the other conjugal Tc^r elements. This homology between the Tc^r Em^r-DOT element and *B. uniformis* 1001 was not required for successful transfer of Tc^r Em^r-DOT to a recipient. *B. thetaiotaomicron* 5482A had no homology by Southern blot analysis to either pD2-T3 or p6T5 (data not shown). Nonetheless, *B. thetaiotaomicron* DOT transferred the Tc^r Em^r-DOT element to both *B. uniformis* 1001 and *B. thetaiotaomicron* 5482A recipients at frequencies of 10⁻⁷ to 10⁻⁶.

Preliminary map of the Tc^r Em^r-DOT element. A preliminary restriction and function map, deduced from the overlapping cosmid clones, is shown in Fig. 5. From the Southern blot shown in Fig. 4, it can be seen that there was no evidence for large deletions in the region covered by the probe, D2T3. Further experiments, in which DNA from *B. uniformis* 1006 was digested with *EcoRI*, *HindIII*, or *HincII* and probed with D2T3 or other cosmid clones, also did not reveal any evidence that large deletions or rearrangements had occurred in the cosmid clones (data not shown). Therefore, the map deduced from the cosmid clones appears to be an accurate map of the element. However, since there are sizable regions in which there are no useful restriction sites, it is not possible to rule out small deletions in these areas.

Endpoints of the element cannot be defined until the internal homology to *B. uniformis* 1001 which is contained on all of the Tc^r clones is located and characterized. There was no internal homology to *B. uniformis* 1001 within two 6-kbp clones that contained only the Em^r genes. However, one of the large Em^r clones, p6E5, had homology to *B. uniformis* 1001 DNA and to the Tc^r Em^r clones (data not shown). This clone had no homology to the clones that contained only the Tc^r gene. We are still trying to determine whether p6E5 contains a true junction fragment.

Preliminary subcloning experiments have placed the Tc^r gene somewhere from 2 kbp left of the *EcoRI* site designated 0, which includes the 1.1-kbp *EcoRV* fragment (Fig. 3 and 4), to 3 kbp to the right. The Em^r gene was more precisely located since we had a specific probe (solid bar in Fig. 5). The hatched area above the map in Fig. 5 indicates the homology to Tn4351 outside the Em^r gene. The transfer region lies within a segment that runs from 2 kbp to the right of *EcoRI* (0) to 20 kbp to the left of this site. p6TE was transferred at very low frequencies, but no transfer of pD2-TE2 (<10⁻⁹) was detected. The differences in the left endpoints of p6TE and pD2-TE2 could be localizing an origin of transfer recognized by an unidentified element in *B. uniformis* 1001.

Four of the six Em^r cosmids were 5 to 10 kbp in size, and

FIG. 4. (A) Autoradiogram of *Bacteroides* strains containing conjugal Tc^r or $Tc^r Em^r$ elements probed with the Tc^r -DOT clone pD2-T3. The total DNA of five clinical isolates containing conjugal Tc^r or $Tc^r Em^r$ elements and their respective *B. uniformis* 1001 transconjugants was digested with *EcoRV* and run on a 1% agarose gel. The Southern blot was probed with the ^{32}P -labeled Tc^r -DOT cosmid clone pD2-T3, which contained 19 kbp of DNA from *B. thetaiotaomicron* DOT. pD2-T3 was isolated from an *E. coli* host to prevent contamination with *B. uniformis* 1001 DNA. The order of the samples (see Table 1) is as follows: *B. fragilis* V479C and *B. uniformis* 1002 (lanes 1 and 1a); *B. fragilis* ERL, *B. uniformis* 1004, and *B. uniformis* 1005 (lanes 2, 2a, and 2b); *B. fragilis* CEST and *B. uniformis* 1003 (lanes 3 and 3a); *B. thetaiotaomicron* DOT and *B. uniformis* 1006 (lanes 4 and 4a); *B. fragilis* 12256 and *B. uniformis* 1008 (lanes 5 and 5a); *B. uniformis* 1001 (lane 6); and *HindIII* λ standards (lane 7). The positions of standards (in kilobase pairs) are indicated on the right, and the expected locations of the 2.4-, 2.1-, and 1.1-kbp *EcoRV* fragments are indicated by arrowheads. (B) Longer exposure of the bottom part of panel A to show the 1.1-kbp segments more clearly.

they covered almost precisely the solid and hatched areas indicated on the map (Fig. 5). Since it is unlikely that 19- to 23-kbp plasmids could be successfully packaged in lambda heads, there seems to be a tendency for specific deletions to occur in this region. The deletions could have occurred in either *E. coli* or *B. uniformis* 1001 hosts. We have also cloned a 6-kbp *Sau3A* fragment from $Tc^r Em^r$ -ERL (*B. uniformis* 1005) on pNJR1 and found that it contains this same Em^r region. The restriction digest pattern appears to be nearly identical to the patterns for Em^r -DOT clones (data not shown). This is not surprising because of the extensive homology seen on Southern blots (Fig. 2). The overlapping cosmids, especially the Tra^+ Tc^r cosmids and the $Tc^r Em^r$ cosmids, place a lower limit on the size of the $Tc^r Em^r$ -DOT element of 44 to 50 kbp.

DISCUSSION

The shuttle cosmid pNJR1 allowed us to clone large segments of *Bacteroides* DNA in *E. coli*. These cloned segments were then mobilized immediately into *Bacteroides* spp. for selection. Many of the cosmid clones were quite unstable in *E. coli*, even in a *recA* strain. Nonetheless, by using a suitably diluted set of cosmid-infected *E. coli* as donors and thus skipping the isolation of individual cosmid clones, we were able to salvage enough cloned DNA to obtain not only the Tc^r and Em^r genes but also large regions of surrounding DNA. In fact, some of our clones contained enough transfer genes to enable them to transfer from *Bacteroides* donors to either *E. coli* or *Bacteroides* recipi-

FIG. 5. Preliminary map of the conjugal $Tc^r Em^r$ -DOT element. A partial restriction map is shown, with an *EcoRI* site designated as 0. The spacing between the marks on the line is 4 kbp. The region containing the three *EcoRV* fragments (2.4, 2.1, and 1.1 kbp) common to four conjugal Tc^r and $Tc^r Em^r$ elements is indicated above the map as Tc^r homologous sequences. The Tc^r gene region, determined by subcloning, is also shown at the top as the solid part of the line. Southern blot analysis localized the Em^r gene to a 1.5-kbp fragment (solid bar), and the region which has homology to the 0.9-kbp *ClaI*-to-*EcoRI* fragment of *Tn4351* is shown as the hatched area. The *HindIII* fragments that appear to be contained by all four of the conjugal $Tc^r Em^r$ elements are indicated above the restriction map. The region of homology probably includes most of the sequences all the way to the *EcoRI* at 0 (Fig. 2). Three of the cosmid clones are shown below the map to indicate their regions of overlap and their phenotypes. The endpoints of $Tc^r Em^r$ -DOT are not known at this time.

ents. Although we cannot rule out the possibility that chromosomal genes are contributing to transfer, the cosmids may have been self-conjugal, because transfer-proficient cosmids were transferred out of *B. thetaiotaomicron* as well as out of *B. uniformis*. We also obtained enough overlapping clones to construct a preliminary map that covers about 50 kbp of the element (Fig. 5). The successful use of the shuttle cosmid to clone the Tc^r and Em^r genes indicates that this strategy might be used to clone other *Bacteroides* genes by complementation of *Bacteroides* mutants and may make complementation mapping possible in *Bacteroides*.

The Tc^r clones contained DNA that hybridized with chromosomal DNA from an isogenic strain of *B. uniformis* which did not contain the element. However, clones covering the same regions that were isolated from *B. thetaiotaomicron* DOT also hybridized with *B. uniformis* chromosomal DNA. Since *B. uniformis* and *B. thetaiotaomicron* have a DNA-DNA homology of only 23% (9), it is unlikely that this homology represents a junction fragment where the element had inserted into the chromosome. Also, this region of homology probably does not represent a highly conserved insertion site for the element that is common to *B. uniformis* and *B. thetaiotaomicron*. For example, the *B. thetaiotaomicron* type strain 5482 does not have DNA that hybridizes with the probes; however, it acts as a recipient for the Tc^r Em^r-DOT element. This region of homology could represent an insertion sequence or a region shared with some as yet undiscovered element in *B. uniformis*. From previous experiments, we had indirect evidence that *B. uniformis* might contain a cryptic conjugal element, because vectors containing a *Bacteroides* plasmid that was too small to be self-mobilizing, pB8-51 in pVAL1, was transferred out of *B. uniformis* 1001 at very low frequencies (10⁻⁷) in the absence of any of the known conjugal elements (30). Further work will be needed to determine whether any of our clones contain a true junction fragment.

In previous experiments, we had shown that the *Bacteroides* Tc^r and Tc^r Em^r elements (including the Tc^r Em^r-DOT element) could mobilize coresident plasmids from *B. uniformis* to *E. coli* (18, 20, 30). We did not know whether the elements transferred themselves into *E. coli*. Since some of our Tc^r clones were transferred from *B. uniformis* to *E. coli*, it is clear that such transfer probably occurs. However, neither the resistance genes nor the transfer genes of the Tc^r Em^r-DOT element function in *E. coli*. This is analogous to the behavior of the IncP plasmid R751, which can mobilize itself into *Bacteroides* spp. but does not replicate there. Also, neither the transfer genes nor the Tp^r gene of an integrated form of R751 functions in *Bacteroides* recipients (17, 20).

Previous work on the conjugal Tc^r and Tc^r Em^r elements that are found in different *Bacteroides* strains indicated that these elements had some similar characteristics. For example, transfer of resistances was not associated with transfer of a plasmid. Also, all of them could mobilize coresident shuttle vectors containing *Bacteroides* plasmid pB8-51 or pBFTM10 to either *Bacteroides* or *E. coli* recipients (18, 30). In most cases the transfer frequency was regulated by the tetracycline (18, 30), and most of the elements could mediate the tetracycline-dependent excision of plasmidlike forms from the *B. uniformis* chromosome (21). However, there was some evidence that the Tc^r elements in different strains were not identical. For example, some elements cotransferred Em^r with Tc^r, whereas others transferred only Tc^r. Transfer of the Tc^r Em^r-12256 element, unlike transfer of the others, was not regulated by tetracycline (11, 30), nor could this

element mediate excision of plasmidlike forms from *B. uniformis* (21). Finally, we were able to show that two elements could coexist in one strain (21). This finding indicated that if the elements were integrated in the chromosome, they did not occupy the same site. Alternatively, if they were large low-copy-number plasmids, they were not incompatible. Southern blot analysis of DNA from strains carrying the different elements, using our *B. thetaiotaomicron* DOT clones as probes, showed that these different elements share regions of homology. These homologous regions include not only regions associated with Tc^r or Em^r but also other regions of the element. Only one element, Tc^r-V479, was clearly distinct from the others. A more detailed restriction and genetic map of the elements will be needed before it will be possible to determine how similar the elements are, but it is clear that they are closely related. Results of Southern hybridization with regions from the Em^r transposon Tn4351 confirm previous reports that the Em^r region on Tc^r Em^r elements does not represent a simple insertion of Tn4351 or some closely related transposon in a Tc^r element. There is no evidence of IS4351 anywhere in the element. Since the insertion elements on the plasmids have been shown to provide the promoter for the Em^r genes (13, 25), the Em^r genes on the Tc^r elements must have different promoters.

The *Bacteroides* conjugal Tc^r elements appear to share some of the phenotypes of the large conjugal Tc^r elements in streptococci. The streptococcal Tc^r elements were first shown to be inserted in the host chromosome in *Streptococcus pneumoniae* (22). A large (67-kbp) conjugal Tc^r element from *Streptococcus agalactiae* was shown to be a conjugal transposon, Tn3951 (8, 27), reminiscent of the 15-kbp Tn916 (4) but much larger and with very few insertion sites. A second 67-kbp conjugal Tc^r element (*Ω*cat tet BM6001) from *S. pneumoniae* has been cloned and analyzed but has not been shown to be a transposon (31). The *Bacteroides* conjugal Tc^r elements are clearly quite large, and it is possible that they, too, are large conjugal transposons. However, no direct evidence for either transposition or insertion in the chromosome has yet been obtained.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI22383 from the National Institute of Allergy and Infectious Diseases and by National Science Foundation grant DMB87-06706.

LITERATURE CITED

- Boyer, H. B., and D. Roulland-Dussoix. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* **41**:459-472.
- Davis, R. W., D. Botstein, and J. R. Roth. 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Davison, J., M. Heusterspreute, N. Chevalier, V. Ha-Thi, and F. Brunel. 1987. Vectors with restriction site banks. V. pJRD215, a wide-host-range cosmid vector with multiple cloning sites. *Gene* **51**:275-280.
- Franke, A. E., and D. B. Clewell. 1981. Evidence for a chromosome-resistance transposon (Tn916) in *Streptococcus faecalis* that is capable of "conjugal" transfer in the absence of a conjugative plasmid. *J. Bacteriol.* **145**:484-502.
- Guiney, D. G., P. Hasegawa, and C. E. Davis. 1984. Expression in *Escherichia coli* of cryptic tetracycline resistance genes from *Bacteroides* R-plasmids. *Plasmid* **11**:248-252.
- Guiney, D. G., P. Hasegawa, and C. E. Davis. 1984. Plasmid transfer from *Escherichia coli* to *Bacteroides fragilis*: differential expression of antibiotic resistance phenotypes. *Proc. Natl. Acad. Sci. USA* **81**:7203-7206.

7. Hwa, V., N. B. Shoemaker, and A. A. Salyers. 1988. Direct repeats flanking the *Bacteroides* transposon Tn4351 are insertion sequence elements. *J. Bacteriol.* **170**:449-451.
8. Inamine, J. M., and V. Burdett. 1985. Structural organization of a 67-kilobase streptococcal conjugative element mediating multiple antibiotic resistance. *J. Bacteriol.* **161**:620-626.
9. Johnson, J. L. 1978. Taxonomy of the *Bacteroides*: deoxyribonucleic acid homologies among *Bacteroides fragilis* and other saccharolytic *Bacteriodes* species. *Int. J. Syst. Bacteriol.* **28**: 245-256.
10. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. Mays, T., C. Smith, R. Welch, C. Delfini, and F. Macrina. 1982. Novel antibiotic resistance transfer in *Bacteroides*. *Antimicrob. Agents Chemother.* **21**:110-118.
12. Odelson, D. A., J. L. Rasmussen, C. J. Smith, and F. L. Macrina. 1987. Review: extrachromosomal systems and gene transmission in anaerobic bacteria. *Plasmid* **17**:87-109.
13. Rasmussen, J. L., D. A. Odelson, and F. L. Macrina. 1986. Complete nucleotide sequence of *ermF*, a macrolide-lincosamide-streptogramin B resistance determinant from *Bacteroides fragilis*. *J. Bacteriol.* **168**:523-533.
14. Robbillard, N. J., F. P. Talley, and M. H. Malamy. 1985. Tn4400, a compound transposon isolated from *Bacteriodes fragilis*, functions in *Escherichia coli*. *J. Bacteriol.* **164**:1248-1255.
15. Saito, H., and K. I. Miura. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta* **72**:619-629.
16. Salyers, A. A., N. B. Shoemaker, and E. P. Guthrie. 1987. Recent advances in *Bacteroides* genetics. *Crit. Rev. Microbiol.* **14**:49-71.
17. Shoemaker, N., C. Getty, J. F. Gardner, and A. A. Salyers. 1986. Tn4351 transposes in *Bacteroides* and mediates the integration of R751 into the *Bacteroides* chromosome. *J. Bacteriol.* **165**:929-936.
18. Shoemaker, N. B., C. Getty, E. P. Guthrie, and A. Salyers. 1986. Two *Bacteroides* plasmids, pBFTM10 and pB8-51, contain transfer regions that are recognized by broad-host-range IncP plasmids and by a conjugative *Bacteroides* tetracycline resistance element. *J. Bacteriol.* **166**:959-965.
19. Shoemaker, N. B., E. Guthrie, A. A. Salyers, and J. F. Gardner. 1985. Evidence that the clindamycin resistance gene of the *Bacteriodes* plasmid pBF4 is on a transposable element. *J. Bacteriol.* **162**:626-602.
20. Shoemaker, N. B., and A. A. Salyers. 1987. Facilitated transfer of IncP R751 derivatives from the chromosome of *Bacteroides uniformis* to *Escherichia coli* recipients by a conjugative *Bacteroides* tetracycline resistance element. *J. Bacteriol.* **169**:3160-3167.
21. Shoemaker, N. B., and A. A. Salyers. 1988. Tetracycline-dependent appearance of plasmidlike forms in *Bacteroides uniformis* 0061 mediated by conjugal *Bacteroides* tetracycline resistance elements. *J. Bacteriol.* **170**:1651-1657.
22. Shoemaker, N. B., M. D. Smith, and W. R. Guild. 1980. DNase resistant transfer of chromosomal *cat* and *tet* insertions by filter mating in pneumococcus. *Plasmid* **3**:80-87.
23. Simon, R., U. Priefer, and A. Pühler. 1983. Research paper. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. *Bio/Technology* **1**:784-791.
24. Smith, C. J. 1985. Characterization of *Bacteroides ovatus* plasmid pBI136 and structure of its clindamycin resistance region. *J. Bacteriol.* **161**:1069-1075.
25. Smith, C. J. 1987. Nucleotide sequence analysis on Tn4551: use of *Erm* FS operon fusions to detect promoter activity in *Bacteroides fragilis*. *J. Bacteriol.* **169**:4589-4596.
26. Smith, C. J., R. A. Welch, and F. L. Macrina. 1982. Two independent conjugal transfer systems operating in *Bacteroides fragilis* V479-1. *J. Bacteriol.* **151**:281-287.
27. Smith, M. D., and W. R. Guild. 1982. Evidence for transposition of the conjugative R-determinants of *Streptococcus agalactiae* B109, p. 109-111. In D. Schlessinger (ed.), *Microbiology—1982*. American Society for Microbiology, Washington, D.C.
28. Speer, B. S., and A. A. Salyers. 1988. Characterization of a novel tetracycline resistance that functions only in aerobically grown *Escherichia coli*. *J. Bacteriol.* **170**:1423-1429.
29. Tally, F. P., D. R. Snyderman, M. J. Shimell, and M. H. Malamy. 1982. Characterization of pBFTM10, a clindamycin-erythromycin resistance transfer factor from *Bacteroides fragilis*. *J. Bacteriol.* **151**:686-690.
30. Valentine, P. J., N. B. Shoemaker, and A. A. Salyers. 1988. Mobilization of *Bacteroides* plasmids by *Bacteroides* conjugal elements. *J. Bacteriol.* **170**:1319-1324.
31. Vijayakumar, M. N., S. D. Priebe, and W. R. Guild. 1986. Structure of a conjugative element in *Streptococcus pneumoniae*. *J. Bacteriol.* **166**:978-984.
32. Welch, R. A., and F. L. Macrina. 1981. Physical characterization of *Bacteroides* R plasmid pBF4. *J. Bacteriol.* **145**:867-872.